General Protocols

Transformation. Get the competent cell (Trans5a, TransGen Biotech) from -80˚C refrigerator and incubate on ice. Once the competent cell has melted, add 2-5μL transforming plasmid. Gently mix with pipette tip and store the tube on ice for 30 minutes. Put the tubes in a preheated 42˚C water bath for exactly 60 seconds, do not shake the tubes. Then rapidly put the tube back on the ice and incubate for at least 3 minutes. Next, add 1mL of LB liquid non-antibiotic medium to each tube and put the tubes to a shake incubator set at 37˚C and 200rp. Shake for 1 hour. Last, the culture is centrifuged for 1 minute at 10,000 ×g, then 900μL culture is discarded. Resuspend bacteria cells in the remaining culture and transfer onto LB plates with corresponding antibiotics. Invert the plates and incubate them at 37˚C. Transformed colonies is expected to be visible in 12-16 hours.

Co-transformation. Co-transformation is similar to transformation. At least two transforming plasmids are added to the competent cell. Agar LB plates contain more than one kind of corresponding antibiotics. If necessary, the amount of antibiotics may be halved.

Colony PCR. Prepare an agar LB medium with corresponding antibiotics first. A PCR reaction mixture is prepared by combining 25μL of 2× Enzyme Master Mix (TsingKe PCR Master Mix), 23μL of ddH_2O and 1μL of forward and reverse primer (10μM), respectively, for each PCR reaction system. Fifty microliters of reaction mix are aliquoted in PCR 8 STRIP TUBES (AXYGEN, PCR-0208-C). Use 10μL white pipette tip to transfer a single bacteria colony to the reaction mixture. After several seconds, take the pipette tip out and gently touch the surface of prepared agar LB medium with the tip, leaving a droplet of liquid. Inverse the plate and incubate at 37˚C for 12 to 16 hours. Transfer the PCR tubes to the thermocycler, start the corresponding program. The PCR product may further be used for agarose gel electrophoresis or DNA sequencing.

Golden Gate. For a 20μL system, ~50ng of each ~3kb DNA vector is combined with insert fragments that are at least two times of the molar concentration of vectors. 2μL of 10× T4 ligase buffer was added. Add 1μL of BsaI and 1μL of T4 ligase to the system, respectively. Finally add ddH_2O up to 20μL. All the enzymes used here are bought from New England Biolabs (NEB). Reaction is performed on a thermocycler using the following conditions: 37˚C for 5 minutes, 16˚C for 10 minutes, cycled for 10 times. Then hold at 37˚C for 15 minutes, 50˚C for 5 minutes, and 65˚C for 10 minutes. Products may be transformed and plated on an agar LB medium with corresponding antibiotics, and colonies shall be visible between 12 to 16 hours.

Gibson Assembly. 15μL of commercially available Gibson Assembly Master Mix was aliquated into PCR tubes. Up to 100ng of each ~6kb of DNA fragments is added. Add ddH_2O to 20μL. Incubate at 50˚C for one hour using a thermocycler. Products may be transformed and plated on an agar LB medium with corresponding antibiotics, and colonies shall be visible between 12 to 16 hours.

Agarose Gel Electrophoresis. Prepare sufficient electrophoresis buffer (1× TAE), and then set the mold with a proper comb on a horizontal section of the bench. Add an appropriate amount of powdered agarose (usually 0.8% ~ 1%) to 1× TAE. Heat the electrophoresis buffer in a microwave oven for around one minute. Make sure that the powder has fully melted, making the solution clear and transparent. Add certain amount of DNA dye (usually 1:10,000) to liquid agarose gel. Mix the gel solution by gentle swirling, and pour it into the mold, waiting for the gel to harden, which usually takes some 20 minutes. Before removing the comb, pour small amount of buffer onto the surface of the gel to prevent air bubbles in the samples slots. Mount the gel onto the electrophoresis task, making sure that samples slots are submerged by buffer solution in the task. Mix 1μL of samples of DNA with 1μL of 6× DNA Loading Buffer and 4μL of ddH_2O. Using a micropipette, load sample mixtures into the slots. 5μL of DNA Marker is also loaded into the slots. Close the lid of the gel tank, attach the electrical leads and make sure that DNA migrates towards positive anode. Usually 125V of electrical potential is applied. Use the distance migrated by bromophenol blue as an indicator of distances migrated by DNA samples. Once DNA samples have migrated for sufficient distance, turn off the electric current and use a UV light to examine the gel and take photos.

Induction of Protein Expression. Add antibiotics (1:1000) and the sample (1:50) in a LB bottle, mark. Incubate overnight on a shake incubator at 37°C for 3~4h until value of OD600 is 0.8. Add IPTG (1:1000) in the bottle. Continue incubate overnight on the shake incubator at 30°C (if the antibiotic is tetX, then 18°C).

Quantification of protein. Protein concentration is determined by the Bradford method. Test the absorbency of graded BAS solution (0.3, 0.2, 0.1, 0.05, 0.025, 0 mg/mL) to build a standard curve by linear regression. Protein specimens are also treated with Bradford method and quantified according to the standard curve.